

Insulin resistance, LDL particle size, and LDL susceptibility to oxidation in pediatric kidney and liver recipients

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Background. Dyslipidemia is common after solid organ transplantation. We have described hypertriglyceridemia in about 50% of our pediatric kidney, and in about 30% of our liver recipients. The aim of the present study was to find out whether this post-transplantation hypertriglyceridemia after pediatric solid organ transplantation is associated with insulin resistance and the occurrence of small, dense low-density lipoprotein (LDL).

Methods. Fifty kidney and 25 liver recipients (aged 4 to 18 years) on triple immunosuppression, and 181 control children participated in the study for an average of 5.3 and 6.4 years after kidney and liver transplantation (range 1 to 11 years), respectively. Homeostasis model assessments for insulin resistance (HOMA) were calculated and fasting lipoprotein lipid profile, apolipoprotein A-I and B concentrations, LDL particle diameter, and indices of LDL susceptibility to copper-induced oxidation determined.

Results. Kidney patients had significantly higher serum total, high-density, and low-density lipoprotein cholesterol, triglyceride, apolipoprotein A-I and B concentrations than liver patients or control subjects ($P < 0.003$ for all). HOMA indices higher than the 95th percentile of Canadian normal children were seen in 50.0% of kidney (of liver 41.2%) recipients younger than 11 years, and in 27.3% of older recipients (of liver 37.5%). Smaller sized LDL or LDL of increased oxidizability was not more frequent in patients than in control children.

Conclusion. Pediatric kidney recipients had significantly higher lipid and insulin concentrations than healthy control children. Combined hyperlipidemia and features of the dysmetabolic syndrome were common in children after kidney and

liver transplantation. However, no small, dense LDL, or LDL prone to oxidation was seen in either group.

Atherosclerosis is the most important cause of death and late graft loss after kidney transplantation (KTx) [1]. Adult liver recipients have a 2.5-fold relative risk for ischemic cardiovascular events, and a 3-fold risk for death [2]. Dyslipidemia is frequent after transplantation (Tx), affecting up to 70% of kidney [3] and 50% of liver recipients [4]. High triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) concentrations, predominance of small low-density lipoprotein (LDL) particles, and elevated insulin and glucose concentrations characterize insulin resistance syndrome [5]. When combined with abdominal obesity, impaired glucose tolerance, or type 2 diabetes mellitus and hypertension, they form a cluster of risk factors with high atherogenic potential [6]. Increased prevalence of insulin resistance [7, 8] and dysmetabolic syndrome has been described in adult KTx patients [9]. After a liver transplantation (LTx) due to cirrhosis, increased first-phase insulin secretion in response to high glucose levels persisted despite LTx normalized pre-Tx insulin resistance [10]. In a large meta-analysis, new onset insulin-dependent diabetes was reported in 13.4% of patients on calcineurin inhibitors (cyclosporine and tacrolimus) after solid organ transplantation [11].

Elevated LDL cholesterol (LDL-C) concentration and small, dense LDL particles prone to oxidation promote atherosclerosis [12–14]. Variation in TG concentration predicted 62% of LDL diameter variation in healthy adults [15]. In addition to environmental factors, the diameter of LDL is genetically determined [16]. Lag time before oxidative modification of LDL in vitro is

Key words: kidney, liver, transplantation, child, lipid, triglyceride, LDL particle size, LDL oxidation, insulin, glucose.

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thought to reflect the total antioxidant status of LDL [17]. HDL provides multiple mechanisms in protection against atherosclerosis (e.g., through reverse cholesterol transport and the ability of HDL to decrease the peroxidation of LDL [18]).

We have reported elevated TG concentrations in 50% and 30% of pediatric kidney and liver recipients on similar triple immunosuppression, respectively, without increased frequency of low HDL-C concentrations. Half of the kidney recipients but only one sixth of the liver recipients and control subjects had elevated total cholesterol (TC) concentration (>5.0 mmol/L). Kidney graft and pretransplantation TC concentration, independent of glomerular filtration rate (GFR) and proteinuria, were risk factors for this hypercholesterolemia [18]. In the kidney recipients, obesity, a high dose of MP, and proteinuria were significant risk factors for dyslipidemia [18]. These findings raised hypotheses that (1) children with solid organ transplantation have increased prevalence of small, dense, and easily oxidative LDL, and hyperinsulinemia, (2) size and oxidizability of LDL are associated with lipoprotein, apolipoprotein B, and insulin concentrations, as well as apolipoprotein E phenotype, (3) the prevalence of small, dense LDL is higher in kidney than in liver recipients.

METHODS

Patients

Patients. Fifty children in the KTx group and 25 children in the LTx group with acceptably functioning grafts took part in a cross-sectional study between September 1997 and April 1999. The inclusion criteria were: age between 3 and 17 years, follow-up time at least 1 year after Tx and glomerular filtration rate (GFR) >40 mL/min/ 1.73m^2 . Mean follow-up time after Tx was 5.3 years (range 1–11 years) and 6.4 (range 1–11 years) in the KTx and LTx groups, respectively. The participation rate was 88.0% in the KTx and 92.6% in the LTx group. Pre-Tx diagnoses in the KTx group were the following: congenital nephrosis ($N = 31$), urethral valve ($N = 5$), nephronophthisis ($N = 2$), polycystic kidney disease ($N = 3$), glomerulonephritis ($N = 2$), prune-belly syndrome ($N = 1$), Alport's syndrome ($N = 1$), mega-ureter ($N = 1$), dysplastic kidney ($N = 1$), bilateral multicystic kidneys ($N = 1$), Denys-Drash syndrome ($N = 1$), and renal insufficiency due to a complication of prematurity ($N = 1$). Pre-Tx diagnoses in the liver group were: tyrosinemia ($N = 7$), hepatitis ($N = 1$), biliary atresia ($N = 9$), hepatoblastoma ($N = 4$), hepatocellular carcinoma ($N = 1$), homozygous familial hypercholesterolemia ($N = 1$), Wilson's disease ($N = 1$), and α -1-antitrypsin deficiency ($N = 1$). None of the patients were hypothyroid, and all were on their usual diet. If there was marked weight gain after Tx, energy restriction and a diet low in saturated fatty acids was advised.

The control group consisted of 181 (112 boys and 69 girls) children without regular medication, acute infection, or inflammatory or metabolic disease [19].

Medication. The immunosuppressive protocols have been described [19]; they included triple therapy with cyclosporine A (CsA) in microemulsion composition, methylprednisolone (MP) given on alternate days, and azathioprine (AZA). Two boys were on triple therapy with tacrolimus instead of CsA in both the KTx and LTx groups. In the LTx group, one girl was on tacrolimus and MP.

Ethics. Parents and children gave written informed consent. The ethical Committees of Tampere and Helsinki University Hospital approved the study.

Methods

Collection of clinical data (kidney function as GFR, creatinine, and diurnal urinary protein; liver function as serum alanine aminotransferase, albumin, total bilirubin, and thromboplastin time; pubertal stage according to Tanner's classification), anthropometric measurements, blood sampling, and analyses were done as described [20]. In short, we expressed measured height and body mass index (BMI) calculated from measured height and weight, as standard deviation score (SDS) calculated according to the equation: (observation — mean observation for age)/SD. SD represents the standard deviation for the normal Finnish population of the same numeric age and gender [21, 22]. We measured blood pressure from the right upper arm of the sitting patient with an electronic sphygmomanometer, and used the mean of the 3 measurements in the analysis.

Lipid and apolipoprotein assays. Blood samples for lipid, cyclosporine trough concentration, insulin, and glucose analyses were taken after a 12-hour overnight fast. Serum and plasma for lipid analyses were immediately separated by centrifugation and stored at -70°C . The serum TG, TC, and HDL-C concentrations, as well as apolipoprotein B (apoB) and A-I (apoA-I) concentrations, were analyzed using Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). We calculated LDL-C concentration by Friedewald's formula [23]. Serum TG did not exceed 4.0 mmol/L in any of the subjects studied. Lp (a) was determined by a radioimmunologic method and expressed in units per liter (U/L; $1\text{ mg/L} = 0.7\text{ U/L}$). Sensitivity of the assay was 17 U/L . Concentrations $>651\text{ U/L}$ (95th percentile of our control children) were considered to be elevated. ApoE phenotype was determined from Tx patients by isoelectric focusing and immunoblotting [20].

Glucose and insulin assays. Blood glucose was analyzed by enzymatic and serum insulin by radioimmunologic methods. We estimated insulin resistance by

homeostasis model assessment [insulin resistance = fast-ing insulin/($22.5e^{-\ln \text{fasting glucose}}$)] [24]. The HOMA index is based on the assumption that normal adults have a HOMA index of 1. We compared the HOMA indices of 9- and 13-year-old Canadian healthy children [25] to our patients divided into subgroups according to gender and age [the median between the 2 cohorts of 9- and 13-year-old Canadians (i.e., <11 years vs. older)]. Oral glucose tolerance tests (OGTT) were performed after a 12-hour fast, and an oral dose of glucose 1.75 g/kg (maximum 75 g) was given. Samples for blood glucose and serum insulin measurements were collected at 0, 30, 60, 90, 120, and 180 minutes. Criteria for hyperinsulinemia were diagnosed as serum fasting insulin exceeding 20 mU/L and/or a peak insulin concentration exceeding 150 mU/L [26]. Impaired glucose tolerance was determined according to WHO criteria: blood glucose 5.6 to 6.0 mmol/L after an overnight fast or 6.7 to 9.9 mmol/L at 120 minutes in the OGTT.

LDL characteristics. The estimation of LDL particle size from EDTA plasma samples was done by nondenaturing gradient gel electrophoresis. In vitro oxidation of LDL was done by copper-induced oxidation of LDL [20]. The maximal storage times for in vitro oxidation of LDL and LDL particle size were 30 and 45 months, respectively.

Food records. Forty-seven of the kidney and 24 of the liver patients completed a 6-day food record, which included 2 weekend days. The patients or their parents were asked to record the type and quantity of the all foods and drinks consumed. The record was checked in an interview with the patient or parent by one of the authors (A.S.). Complementary data from another parent or caretaker were in some cases elicited by telephone (e.g., in cases when the type of fat used in preparing the dish was not reported). Data on day-care meals and school lunches were checked by telephone from the kitchen personnel of the day-care centers and schools. Simple models of foods (e.g., different-sized potatoes), volume, and household measures and pictures of food amounts and pictures of dietary fat packages on the market at the time were used in checking the food record data. Of the controls, 178 completed a 3-day food record, which included 1 weekend day. Food record data were analyzed with a Finnish nutrient software program (Nutrica 3.1 Fin, The Social Insurance Institution of Finland, Turku, Finland).

Statistical analysis

Normality of distributions was tested by one-sample Kolmogorov-Smirnov goodness of fit test. If the distribution was skewed, we used a logarithmic scale in appropriate cases, but described the data as untransformed values. When the distribution of TG was skewed, we displayed the mean of TG distribution as a geometric mean (the antilogarithm of the mean of the log transformed

distribution). We presented normally distributed continuous variables as mean, range, and confidence intervals, and discrete or skewed distributed variables as median, lower (Q_1), and upper quartile (Q_3). We presented MP dose as daily dose, which equals the dose on alternate days divided by 2. We tested differences in means between groups with t test or analysis of variance (ANOVA, Bonferroni correction for multiple comparisons) for normally distributed continuous variables, or Mann-Whitney or Kruskal-Wallis test for skewed or discrete variables. The significance of difference in the frequency distribution was tested by chi-square test. In cases where the frequencies in the cells were low, Fisher exact test was used instead. We calculated Pearson correlation coefficients for normally distributed variables and Spearman correlation coefficients for variables of skewed or discrete distributions. We presented a maximum of 4 most significant univariate correlation coefficients. In the tables, we tested all differences between the groups and presented those that were statistically significant. We considered two-sided P values less than to be 0.05 statistically significant.

We evaluated the determinants of TG concentration, LDL susceptibility to in vitro oxidation (lag time and oxidation rate) and LDL particle size by multivariate linear regression analysis, and used forward stepwise method. Normally distributed variables were included as continuous, and variables of skewed or discrete distributions as dichotomized. We did analyses separately for both Tx groups. We included a maximum of 7 possible risk factors first in 4 different blocks (Table 1). In all first blocks for LDL characteristics, HDL-C and TG concentrations were included. Similarly, in all first blocks for TG, HDL-C concentration and HOMA index were included. Significant variables from the first blocks were then included in the final block. The variables in the model did not show a strong correlation ($r < 0.4$). A variable was included in the multivariate model if its significance was < 0.05 , and removed if the significance was > 0.1 . Computations were carried out using SPSS for Windows version 10.1 (SPSS, Inc., Chicago, IL, USA).

RESULTS

The patient and control groups were similar in age, relative weight, and distributions of pubertal stage (Table 2). The kidney recipients had higher systolic blood pressure than the controls ($P < 0.001$). There were differences in the intake of carbohydrates between the groups; 41.7% ($N = 10$) of the liver recipients reported high carbohydrate intake ($>55\%$ of energy) compared to 10.6% ($N = 5$) of the kidney recipients, and 22.5% ($N = 40$) of the controls ($P = 0.011$) (Table 2). Medications are given in Table 3. The kidney function of kidney recipients was inferior to that of liver recipients (GFR: $P <$

Table 1. Block division of multivariate linear regression models for triglyceride, LDL particle size, and variables of LDL oxidation

Characteristics of patients
Gender (0 = boy, 1 = girl)
Time since transplantation <i>years</i>
Body mass index standard deviation score
Pubertal stage (0 = prepubertal, 1 = pubertal or postpubertal)
Indication for transplantation (kidney: 0 = NPHS1 vs. 1 = other; liver: 0 = liver failure vs. 1 = other)
HDL-C <i>mmol/L</i>
Triglyceride <i>mmol/L</i> (logarithmic scale, in the model for triglyceride HOMA included)
Medication and blood pressure
Blood cyclosporine trough level $\mu\text{mol/L}$
Methylprednisolone dose (0 = two lowest tertiles, 1 = the highest tertile)
Use of growth hormone (0 = no, 1 = yes)
Use of antihypertensives (in the model for kidney recipients only; 0 = no, 1 = yes)
Systolic blood pressure <i>mm Hg</i>
HDL-C <i>mmol/L</i>
Triglyceride <i>mmol/L</i> (logarithmic scale, in the model for triglyceride HOMA included)
Kidney and liver function
Graft (in the model for kidney recipients only, 0 = cadaver, 1 = living related donor)
Glomerular filtration rate [0 = two highest tertiles, 1 = the lowest tertile (in the kidney group <48.6 mL/min/1.73m ² , in the liver group <65.4 mL/min/1.73m ²)]
Thromboplastin time (0 = two highest tertiles, 1 = the lowest tertile)
Urinary protein (in the model for kidney recipients only; 0 = “≤200 mg/day,” 1 = “>200 mg/day”)
HDL-C <i>mmol/L</i>
Triglyceride <i>mmol/L</i> (logarithmic scale, in the model for triglyceride HOMA included)
Lipids, insulin resistance, and diet
Low-density lipoprotein particle size (not included the model for triglyceride)
Homeostasis model assessment index for insulin resistance [24] (0 = two lowest tertiles, 1 = the highest tertile)
Apolipoprotein E phenotype (E3/E4 or E4/E4, 0 = no, 1 = yes)
Intake of carbohydrate (percentage of total energy; 0 = “<55 E%,” 1 = “≥55 E%”)
Intake of saturated fat (percentage of total energy)
HDL-C <i>mmol/L</i>
Triglyceride <i>mmol/L</i> (logarithmic scale, in the model for triglyceride HOMA included)

Abbreviations are: NPHS1, congenital nephrotic syndrome; HDL-C, serum high-density lipoprotein cholesterol concentration. Dependent variables were not included in the model as independent variables.

Table 2. Clinical characteristics of 75 kidney or liver transplant recipient children and 181 controls

	Kidney (N = 50)	Liver (N = 25)	Control (N = 181)
Gender, Male/Female, N	33/17	15/10	112/69
Age <i>years</i> [median (range)]	10.8 (4.3–17.2)	9.3 (3.9–17.9)	9.1 (3.2–18.7)
Median age at Tx <i>years</i> [median (range)]	3.2 (1.1–15.3) ^a	2.0 (0.4–16.3)	
Height SDS, mean (CI)	−1.3 (−1.6; −1.0) ^b	−1.7 (−2.1; −1.3)	0.2 (0.0; 0.3)
BMI SDS, mean (CI)	0.3 (−0.1; 0.6)	0.5 (−0.1; 1.1)	0.3 (0.1; 0.5)
Pubertal stage (Tanner’s classification)			
Pre-pubertal% (N)	48.0 (24)	60.0 (15)	61.8 (112)
Pubertal% (N)	42.0 (21)	32.0 (8)	29.8 (54)
Post-pubertal% (N)	10.0 (5)	8.0 (2)	8.3 (15)
Systolic blood pressure, mmHg, mean (CI)	119 (115; 122) ^c	115 (109; 120)	109 (107; 110)
Diastolic blood pressure, mmHg, mean (CI)	65 (61; 68)	69 (69; 74)	65 (63; 66)
Diet (N = 47/24/178)			
Energy intake, kJ, median (Q ₁ ; Q ₃)	7.2 (5.6; 8.8)	7.0 (6.1; 7.6)	7.5 (6.4; 8.8)
Carbohydrates, E%, mean (CI)	49.0 (47.6; 50.5) ^d	53.2 (51.0; 55.4)	50.7 (49.8; 51.5)
Fat, E%, mean (CI)	34.1 (32.8; 35.4)	31.2 (29.1; 33.3)	33.2 (32.5; 33.9)
Saturated fat, E%, mean (CI)	14.5 (13.8; 15.2)	13.2 (11.8; 14.6)	14.1 (13.7; 14.5)
Dietary cholesterol, mg, median (Q ₁ ; Q ₃)	195 (157; 261)	207 (157; 254)	221 (174; 278)

^a $P = 0.014$

^b $P < 0.001$; kidney group vs. controls and liver group vs. controls, $P < 0.001$

^c $P < 0.001$; kidney group vs. controls, $P < 0.001$ (liver group vs. controls, $P = 0.074$)

^d $P = 0.012$; kidney vs. liver $P = 0.01$

STATISTICS: chi square test, Kruskal-Wallis test, ANOVA (Bonferroni correction for multiple comparisons), E% = percent of energy.

NOTES: Part of the control data has been published [19, 20].

0.001; creatinine: $P < 0.001$) (Table 4). Liver recipients had higher serum alanine aminotransferase concentration ($P < 0.001$) and lower serum thromboplastin time ($P = 0.019$) than kidney recipients. Kidney recipients had

higher serum TC ($P < 0.001$) and LDL-C concentrations ($P = 0.002$), and also higher HDL-C ($P < 0.001$) and apoA-I concentrations ($P = 0.001$), than liver recipients or controls (Table 5).

Table 3. Medication of 75 kidney or liver transplant recipient children

	Kidney (N = 50)	Liver (N = 25)
Cyclosporine A mg/kg/day, median (Q ₁ ; Q ₃)	5.4 (3.7; 6.8)	4.6 (3.6; 5.3)
Blood cyclosporine trough concentration µg/L, median (Q ₁ ; Q ₃)	94 (78; 119)	102 (73; 134)
Methylprednisolone mg/kg/day, median (Q ₁ ; Q ₃)	0.09 (0.07; 0.12) ^a	0.07 (0.06; 0.10)
Azathioprine mg/kg/day, median (Q ₁ ; Q ₃)	1.3 (1.2; 1.4) ^b	1.2 (1.1; 1.3)
Antihypertensives % (N)	28.0 (14) ^c	8.0 (2)
β-blockers or diuretics % (N)	6.0 (3)	4.0 (1)
Growth hormone treatment % (N)	16.0 (8)	24.0 (6)
Hydrocortisone substitution % (N)	20.0 (10)	40.0 (10)

Q₁, lower quartile; Q₃, upper quartile. Mann-Whitney test and chi-square test were used.

^a *P* = 0.014.

^b *P* = 0.001.

^c *P* = 0.046.

Insulin, glucose, and TG

An increased TG concentration (>1.5 mmol/L) was seen in 40.0% (*N* = 20) of the KTx, in 16.0% (*N* = 4) of the LTx patients, and in 7.2% (*N* = 13) of controls (*P* < 0.001). Hyperinsulinemia, according to either high fasting or peak insulin concentration >150 mU/L during OGTT, was seen in 10.0% (*N* = 5) of the kidney and in 16.0% (*N* = 4) of the liver recipients (Table 6). In the KTx group, HOMA indices higher than the 95th percentile of Canadian normal population were observed in 50.0% (*N* = 14) of the younger, and in 27.3% (*N* = 6) of older children, respectively, indicating a high prevalence of insulin resistance. In the LTx group, the corresponding figures were 41.2% (*N* = 7) and 37.5% (*N* = 3). Both an increased TG concentration and a high HOMA were present in 22.0% (*N* = 11) of KTx and 4.0% (*N* = 1) of LTx patients. Impaired glucose tolerance was seen in 20.0% (*N* = 10) and 32.0% (*N* = 8) of patients in KTx and LTx groups, respectively. One girl in the LTx group was classified as prediabetic with blood glucose of 10.4 mmol/L at 2 hours. In KTx patients, TG concentration correlated with HDL-C (*r* = -0.381, *P* = 0.006) and BMI SDS (*r* = 0.369, *P* = 0.008), while no such correlations were seen in the LTx group. In both Tx groups, fasting insulin correlated with systolic blood pressure (kidney: *r* = 0.379, *P* = 0.007; liver: *r* = 0.565, *P* = 0.003). In the final multivariate regression model, variation in HDL-C, BMI SDS, and urinary protein explained 30.8% of variation in TG in the KTx group (Table 7).

LDL particle size

KTx patients had larger LDL particle size than controls (*P* < 0.001) (Table 5).

In the KTx group, variation in HDL-C concentration explained 40.7% of variation in LDL particle size,

Table 4. Graft function in 75 kidney or liver transplant recipient children studied for dyslipidemia

	Kidney (N = 50)	Liver (N = 25)
GFR mL/min/1.73m ² , mean (CI) (range)	60 (56; 65) ^a (41–106)	84 (71; 96) (41–165)
Creatinine µmol/L, median (Q ₁ ; Q ₃)	96 (80; 109) ^a	58 (48; 75)
Alanine aminotransferase U/L, median (Q ₁ ; Q ₃)	13 (12; 19) ^a	32 (14; 61)
Albumin g/L, mean (range)	37 (24–45) ^b	39 (31–45)
Total bilirubin µmol/L, median (Q ₁ ; Q ₃)	ND	10 (7; 15)
Thromboplastin time%, mean (CI)	102 (95; 109) ^c	88 (80; 96)

ND, not determined; Q₁, lower quartile; Q₃, upper quartile. *t* test and Mann-Whitney test were used.

^a *P* < 0.001.

^b Serum albumin concentration of 9 kidney patients.

^c *P* = 0.019.

whereas that in TG concentration explained 12.8% (Table 8). In the LTx and control groups, variation in HDL-C concentration explained 36.1% and 17.9% of variation in LDL diameter, respectively. Univariate correlation was seen between LDL particle size and HOMA index for insulin resistance (*r* = 0.321, *P* = 0.023) in the KTx group, and in the LTx group between LDL particle size and BMI SDS (*r* = 0.510, *P* = 0.011). In the final multivariate regression model, variation in HDL-C concentration and pubertal stage explained 46.5% of variation in the LDL particle size in the KTx group (Table 7), and variation in HDL-C concentration and BMI SDS explained 59.8% of variation in the LDL particle size in the LTx group.

LDL susceptibility to oxidation

LDL oxidation rate and maximum amount of formed dienes were highest in the KTx group and lowest in the LTx group (*P* = 0.001, Table 5). In kidney patients, lag time correlated positively with HDL-C (*r* = 0.290, *P* = 0.05), and negatively in the liver patients (*r* = -0.539, *P* = 0.005) (Table 8). In the liver group, oxidation rate correlated with TC concentration (*r* = 0.724, *P* < 0.001). Oxidation rate was also associated with maximum amount of dienes formed in both groups (kidney group: *r* = 0.748, *P* < 0.001; liver group: *r* = 0.864, *P* < 0.001).

DISCUSSION

Risk for cardiovascular diseases is significantly elevated in kidney and liver recipients [1, 2]. This risk is a combination of preexisting factors, such as the underlying kidney disease leading to specific metabolic abnormalities and transplantation, and the factors associated with transplantation, especially graft function and immunosuppressive therapy [1, 27]. In our study, kidney transplant recipients had significantly higher TC, LDL-C, and apoB concentrations than liver recipients and controls.

Table 5. Serum lipids and lipoproteins and characteristics of LDL particles in 50 kidney and 25 liver transplant recipient children and 181 controls

	Kidney (N = 50)	Liver (N = 25)	Control (N = 181)	P value
Lipoprotein values				
TC <i>mmol/L</i> , mean (CI)	4.99 (4.71; 5.28)	4.25 (3.83; 4.68)	4.18 (4.07; 4.29)	<0.001 ^{a,b}
LDL-C <i>mmol/L</i> , mean (CI)	2.70 (2.48; 2.92)	2.16 (1.84; 2.48)	2.39 (2.30; 2.48)	0.002 ^{a,b}
HDL-C <i>mmol/L</i> , mean (CI)	1.62 (1.52; 1.72)	1.56 (1.37; 1.74)	1.42 (1.38; 1.47)	<0.001 ^a
TG <i>mmol/L</i> , geometric mean (CI)	1.34 (1.17; 1.52)	1.09 (0.93; 1.28)	0.72 (0.67; 0.77)	<0.001 ^{a,c}
HDL-C/TC%, mean (CI)	33.2 (31.1; 35.2)	36.8 (33.4; 40.2)	34.6 (33.5; 35.7)	NS
ApoA-I <i>g/L</i> , mean (CI)	1.53 (1.45; 1.60)	1.46 (1.34; 1.59)	1.39 (1.35; 1.42)	0.001 ^a
ApoB <i>g/L</i> , mean (CI)	0.83 (0.77; 0.89)	0.70 (0.62; 0.77)	0.73 (0.71; 0.76)	0.001 ^{a,b}
Lp (a) <i>U/L</i> , median (Q ₁ ; Q ₃) (range)	65 (21; 143) (<17; 673)	67 (32; 161) (<17; 535)	85 (27; 238) (<17; 1157)	NS
Apolipoprotein E3/E4 or E4/E4 phenotype% (N)	28.0 (14)	24.0 (6)	ND	NS
LDL characteristics				
Diameter of LDL <i>nm</i> , mean (CI) ^d	26.9 (26.8; 27.1)	26.8 (26.6; 26.9)	26.6 (26.5; 26.7)	<0.001 ^a
Diameter of LDL <25.6 <i>nm</i> % (N)	2.0 (1)	0.0 (0)	1.1 (1)	<0.001
Diameter of LDL 25.6< <i>x</i> <26.5 <i>nm</i> ,% (N)	18.0 (9)	16.7 (4)	49.5 (47)	
Diameter of LDL 26.5< <i>x</i> <27.2 <i>nm</i> ,% (N)	42.0 (21)	70.8 (17)	38.9 (37)	
Diameter of LDL>27.2 <i>nm</i> ,% (N)	38.0 (19)	12.5 (3)	10.5 (10)	
Lag time <i>minutes</i> , mean (CI) ^e	78 (76; 81)	76 (71; 80)	77 (75; 78)	NS
Oxidation rate $\mu\text{mol/min}$, mean (CI) ^e	0.44 (0.43; 0.45)	0.40 (0.37; 0.43)	0.43 (0.42; 0.44)	0.001 ^{b,c}
Maximum amount of dienes formed <i>nmol/mg</i> , mean (CI) ^e	568 (553; 584)	522 (496; 549)	547 (538; 556)	0.001 ^{a,b}

Abbreviations: TC, serum total cholesterol concentration; CI, 95% confidence interval for mean; LDL-C, serum low density lipoprotein concentration; HDL-C, serum high-density lipoprotein cholesterol concentration; TG, serum triglyceride concentration; ApoA-I, serum apolipoprotein A-I concentration; ApoB, serum apolipoprotein B concentration; Lp (a), serum lipoprotein (a) concentration; NS, not significant; Q₁, lower quartile, Q₃, upper quartile; LDL, low density lipoprotein; ND, not determined. ANOVA (Bonferroni correction for multiple comparisons), Kruskal-Wallis test, and chi-square test were used. Part of the control data has been published [19, 20].

^aKidney group vs. controls, *P* < 0.05.

^bKidney vs. liver group, *P* < 0.05.

^cLiver group vs. controls, *P* < 0.05.

^dLDL particle size was analyzed for 24 children in the liver recipient, and for 95 children in the control group.

^eLDL susceptibility to in vitro oxidation was analyzed for 46 children in the kidney recipient, and for 88 children in the control group.

Table 6. Serum insulin and homeostasis model assessment index for insulin resistance in 50 kidney and 25 liver transplanted children and 181 controls

	Kidney (N = 50)	Liver (N = 25)	Control (N = 181)	P value
Insulin				
Insulin <i>mU/L</i> , median (Q ₁ ; Q ₃)	10 (7; 16)	9 (5; 15)	5 (3; 7)	<0.001
Prepubertal, median (Q ₁ ; Q ₃) (N = 24/15/112)	8 (4; 11)	6 (4; 11)	4 (3; 5)	<0.001
Pubertal, median (Q ₁ ; Q ₃) (N = 21/8/54)	14 (10; 19)	13 (5; 36)	7 (5; 10)	<0.001
Postpubertal, median (Q ₁ ; Q ₃) (N = 5/2/15)	9 (8; 14)	(9; 18)	6 (5; 14)	NS
Frequency of high fasting insulin% (N)	10.0 (5)	12.0 (3)	1.7 (3)	NS
Frequency of hyperinsulinemia during OGTT% (N)	3.9 (2)	8.0 (2)		NS
Insulin resistance				
HOMA insulin resistance, median (Q ₁ ; Q ₃)	2.2 (1.4; 3.3)	1.8 (0.9; 2.9)		NS
Prepubertal, median (Q ₁ ; Q ₃) (N = 24/15)	1.6 (0.8; 2.4)	1.2 (0.9; 2.2)		NS
Pubertal, median (Q ₁ ; Q ₃) (N = 21/8)	2.9 (2.2; 4.4)	2.9 (2.0; 7.0)		NS
Postpubertal, median (Q ₁ ; Q ₃) (N = 5/2)	1.7 (1.6; 2.9)	2.7 (1.7; 3.7)		NS

Abbreviations: Q₁, lower quartile; Q₃, upper quartile; NS, not significant; OGTT, oral glucose tolerance test; HOMA, insulin resistance index according to homeostasis model assessment [24]. Kruskal-Wallis test and chi-square test were used. Part of the control data has been published [19, 20].

Both kidney and liver recipients had higher serum triglyceride concentrations than the controls. Despite this dyslipidemia, small, dense, and easily oxidative LDL was not seen in either the kidney or in the liver Tx group. Signs of dysmetabolic syndrome were seen in both solid organ recipient groups because a high HOMA index, indicating insulin resistance, was found in 50.0% of the young kidney recipients (<11 years of age) and in 41.2% of the liver recipients. Impaired glucose tolerance was found in 20.0% and 32.0% of kidney and liver recipients, respectively.

Hyperlipidemia, hypertension, dysmetabolic syndrome, and new-onset insulin dependent diabetes are well-known risk factors for cardiovascular diseases after transplantation, but also risk factors for graft failure because they may promote vascular changes in transplanted organs and, thereby, enhance the development of chronic rejection [1, 9, 28, 29]. Insulin-resistant individuals usually have an elevated concentration of TG, a decreased concentration of HDL-C, and preponderance of small, dense LDL [30]. Forty percent of the kidney recipients had an elevated TG concentration. As expected,

Table 7. Multivariate linear regression models for 50 kidney and 25 liver transplant recipients

Organ group	Dependent variable	Block	Independent variable	First blocks				Final block			
				B	Std. error	P	R ²	B	Std. error	P	R ²
Kidney	TG	A	HDL-C ^a	-0.44	0.18	0.019	0.234	-0.45	0.17	0.013	0.308
			BMI SDS	0.11	0.05	0.024		0.11	0.05	0.031	
	LDL particle size	A	HDL-C	-0.53	0.17	0.004	0.246				
			Urinary protein	0.56	0.24	0.026		0.53	0.24	0.031	
			Pubertal stage	1.07	0.17	<0.001	0.465	1.07	0.17	<0.001	0.465
Liver	Lag time for LDL oxidation (N = 46)	B	HDL-C ^b	8.57	3.35	0.014	0.210	7.15	3.32	0.037	0.179
	LDL particle size (N = 24)	A	Use of growth hormone	6.33	2.92	0.036		6.66	2.98	0.031	
			HDL-C ^a	0.48	0.11	<0.001	0.598	0.48	0.11	<0.001	0.598
	Lag time for LDL oxidation	A	BMI SDS	0.13	0.04	0.002		0.13	0.04	0.002	
			HDL-C ^c	-16.93	3.72	<0.001	0.542	-7.53	3.42	0.038	0.590
	Oxidation rate		Time since transplantation	1.96	0.63	0.005					
			Indication for LTx: failure vs. other	8.19	3.88	0.047					
		B	Use of growth hormone	13.60	3.93	0.002	0.363	14.24	3.56	0.001	
		B	Methylprednisolone dose	-0.06	0.02	0.018	0.441				
		D	HDL-C ^a	0.06	0.02	0.023		0.08	0.03	0.005	0.302
			HDL-C	0.09	0.02	0.001	0.462				
			Intake of saturated fat	-0.01	0.003	0.037					

Abbreviations: B, beta (i.e., the unstandardized coefficients of the estimated regression model); std. error, standard error of the unstandardized coefficients of the estimated regression model; P, P value of the significance; R², the proportion of variation in the dependent variable explained by the regression model; TG, serum triglyceride concentration; HDL-C, serum high-density lipoprotein cholesterol concentration; BMI SDS, body mass index standard deviation score, LDL, low-density lipoprotein; LTx, liver transplantation. Linear regression was performed. The setting and block division of variables are presented in Table 1.

^a HDL-C was significant in all first blocks. Blocks with HDL-C as the only significant variable are not presented.

^b HDL-C was also significant in block D.

^c HDL-C was also significant in blocks C and D.

Table 8. Univariate linear regression models for characteristics of LDL with lipids, lipoproteins and apolipoproteins as independent variables in kidney and liver transplantation groups and controls

Dependent variable	Independent variable	Kidney transplant group				Liver transplant group				Control group			
		B	Std. error	P	R ²	B	Std. error	P	R ²	B	Std. error	P	R ²
LDL particle size (N = 50/24/95)	HDL-C mmol/L	1.01	0.18	<0.001	0.407	0.50	0.14	0.002	0.361	0.66	0.15	<0.001	0.179
	TG mmol/L	-0.41	0.16	0.011	0.128					-0.29	0.10	0.006	0.077
	ApoA-I g/L	1.02	0.28	0.001	0.223	0.62	0.22	0.010	0.263	0.61	0.21	0.005	0.082
	ApoB g/L	-0.77	0.38	0.047	0.081					-0.63	0.28	0.030	0.050
Lag time for LDL oxidation (N = 46/25/88)	TC mmol/L					-5.28	1.85	0.009	0.261				
	LDL-C mmol/L					-6.29	2.55	0.021	0.210				
	HDL-C mmol/L	6.99	3.47	0.050	0.084	-12.54	4.09	0.005	0.291				
	TG mmol/L												
	ApoA-I g/L	9.84	4.74	0.044	0.091	-14.82	6.39	0.030	0.189				
	ApoB g/L					-26.14	11.04	0.027	0.196				

Abbreviations: B, beta (i.e., the unstandardized coefficients of the estimated regression model); std. error, standard error of the unstandardized coefficients of the estimated regression model; P, P value of the significance; R², the proportion of variation in the dependent variable explained by the regression model; LDL, low density lipoprotein; HDL-C, serum high-density lipoprotein cholesterol concentration; TG, serum triglyceride concentration; ApoA-I, serum apolipoprotein A-I concentration; ApoB, serum apolipoprotein B concentration; TC, serum total cholesterol concentration. Linear regression was performed.

variation in TG concentration showed an association with variation in HDL-C concentration, obesity, and proteinuria, and also a univariate association with LDL diameter. Thus, the lipid associations typical of dysmetabolic syndrome were seen in our kidney recipients. The underlying causes of hypertriglyceridemia can be treated, at least to some extent, by adequate weight control and by minimizing factors leading to proteinuria.

Corticosteroids are associated with combined hyperlipidemia, hyperinsulinemia, and hypertension in non-

transplant patients [31] and transplant recipients [32]. Lemieux et al showed that kidney recipients on prednisone had higher serum insulin and apoB concentrations, and female kidney recipients also more weight than the same patients after cessation of prednisone [8]. According to Ekstrand [7], the most important mechanism associated with steroid-induced insulin resistance is decreased activity of glycogen synthase. This, together with decreased insulin secretion, predisposes to glucose intolerance [7]. Glucocorticoids might increase lipid production

and impair lipid catabolism in the liver [8, 31]. In our study, both kidney and liver recipients were on similar low-dose, every-other-day corticosteroid regimens, the average dosage being slightly higher in kidney than in liver recipients. Although no dose-dependent influence was seen, the features of insulin resistance found in both of our Tx groups could have been due to corticosteroid therapy.

Small, dense LDL is associated with increased risk of coronary heart disease [14]. Hypertriglyceridemia, insulin resistance, and hypertension have been reported to be associated with increased prevalence of small, dense LDL [33]. Our kidney recipients especially had risk factors for increased prevalence of small, dense LDL. Thus, it was unexpected that neither small, dense LDL nor LDL prone to *in vitro* oxidative modification existed more frequently in our patients than controls. In fact, kidney recipients seemed to have rather increased frequency of large LDL diameters. The rare occurrence of small, dense LDL in our patients may be explained by age, as full penetration has been reported in only subjects over 20 years of age or genetic predisposition to larger-sized LDL [16]. Nevertheless, small, dense LDL was rare in our patients. We wanted to model these lipoproteins in order to see whether the risk factors usually reported to influence the prevalence of small, dense LDL controlled the variation of LDL diameters in our patients. In our KTx patients, LDL diameter showed an independent positive association with HDL-C, and an inverse univariate association with TG concentration, as previously reported in adults with or without Tx [14, 34]. In previous reports, TG concentration has typically been the strongest determinant of LDL diameter, but in our study HDL-C concentration was the most important univariate lipid determinant, explaining up to 40.7% of variation of LDL diameter in our kidney patients. Due to the metabolic relationship between a high TG and a low HDL-C concentration (increased catabolic rate of triglyceride-rich HDL particles resulting in low HDL-C concentration) [35], the association of LDL diameter with HDL-C concentration probably reflected some persisting influence of the hypertriglyceridemia. Our kidney recipients had higher concentrations of HDL-C and apoA-I than our controls, probably because glucocorticoid therapy increases HDL-C concentration and apoA-I messenger RNA levels [36], and reduces plasma cholesterol ester transfer protein activity [8]. This might indicate efficient reverse cholesterol transport, which supports the catabolism of triglyceride-rich lipoproteins decreasing the formation of small, dense LDL [37, 38]. Contrary to our initial hypothesis and a previous report on Japanese children [39], insulin resistance, seen especially in our patients with ongoing puberty, and obesity were not associated with small, dense, but rather with large LDL diameters in our patients. Thus, in pediatric kidney or liver recipients, the cardiovascular risk did

not seem to be mediated through small, dense, and easily oxidative LDL.

According to the 6-day food records, the amount of fat in the diet (percent of energy) did not differ statistically significantly between the groups, but high relative carbohydrate intake was more common in liver than in kidney patients. Previously, high carbohydrate diet has been shown to be associated with increased concentration of TG and prevalence of small, dense LDL [40]. These associations of high carbohydrate diet were not seen in our patients, probably due to the complex influence of diet on lipoprotein values, and also due to the multifarious nature of dyslipidemia in solid organ recipients.

Previously, in kidney transplant patients receiving CsA, a shorter lag time for LDL oxidation has been seen [34, 41, 42]. In our patients, we saw no pro-oxidant effect of CsA. Our patients used CsA in microemulsion composition containing DL- α tocopherol, which might act as an antioxidative agent [43]. Still, *in vitro* studies do not suggest CsA to be a direct pro-oxidant [44]. *In vivo*, HDL protects LDL against lipid peroxidation via various mechanisms, as acting as a reservoir for lipid peroxides and retarding LDL oxidation by HDL-associated enzymes [18]. Our kidney and liver patients and controls were different in associations of cholesterol with lag time. However, patients with GH treatment had a longer lag time in both Tx groups. These findings remain to be explained. With regard to lipid metabolism, kidney and liver are metabolically different organs because the liver plays an essential, well-known role in the synthesis and storage of lipids, while deteriorating kidney function leads to alterations in concentrations and compositions of lipids, but the role of kidney in the regulation of lipid homeostasis is not clearly known.

In our study, multivariate associations were assessed first in blocks that let us include more independent variables in analysis (Table 1). We tried to group possible related risk factors in a block (e.g., kidney and liver function). However, in the block of patient characteristics, the variables were only weakly related. This setting did not allow all interactions between the variables to be studied, and the final model may not show the best possible subset of all variables in first blocks.

CONCLUSION

Higher TG and insulin concentrations were found in kidney and liver transplant recipients compared to controls. Also, features of dysmetabolic syndrome, except increased prevalence of small, dense, and easily oxidative LDL, were seen in both organ recipient groups. HDL-C concentration was the most important determinant for LDL particle size in all groups. These preliminary findings in a limited number of pediatric kidney and liver recipients should be followed up with a larger patient group

in the future. Because dyslipidemia, hypertension, dysmetabolic syndrome, and new-onset insulin-dependent diabetes are risk factors for future cardiovascular disease, the occurrence of these risk factors should be minimized, and be a prime consideration in any management strategy to reduce the mortality and morbidity of cardiovascular diseases in pediatric transplant recipients.

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